AMENDMENT NO. 1 FEBRUARY 2016
TO
IS 1007 : 1984 SPECIFICATION FOR CUSTARD POWDER

(Second Revision)

(*Page* 4, c*lause* **3.2**) — Insert the following at the end:

'Starch content shall be determined by the method given in Appendix B'

(*Page* 9, *Appendix* A) — Insert the following new Appendix B after Appendix A:

'APPENDIX B

(*Clause* 3.2)

DETERMINATION OF STARCH CONTENT

B-0 GENERAL

Starch is a polysaccharide made up of glucose units. Custard powder is first washed with alcohol to remove already present reducing sugar and the reducing sugar free custard powder sample containing starch is hydrolyzed using acid to break the starch molecule into glucose. The glucose (Reducing sugar) is determined either by colorimetric method or by titration. Starch is calculated by the formula given below:

Percent starch = Percent reducing sugar (Glucose) x 0.90

B-1 REAGENTS

B-1.1 95 Percent Alcohol — Take 95 ml of alcohol and dilute to 100 ml with distilled water.

B-1.2 50 Percent Alcohol — Take 50 ml of alcohol and dilute to 100 ml with distilled water.

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B-1.3 10 Percent Alcoholic Solution of Alpha Naphthol — Weigh 10 g alpha naphthol and dissolve in absolute alcohol and finally make up the volume to 100 ml using absolute alcohol.

B-1.4 Concentrated Sulphuric Acid

B-1.5 Concentrated Hydrochloric Acid

B-1.6 Sodium Hydroxide (1 N) — Dissolve 4 g of sodium hydroxide in distilled water and raise the volume to 100 ml with distilled water.

B-1.7 Phenolphthalein Indicator — Dissolve 1 g of phenolphthalein in 100 ml of absolute alcohol.

B-2 APPARATUS

B-2.1 Electronic Balance

B-2.2 Conical Flask

B-2.3 Beaker

B-2.4 Burette

B-2.5 Centrifuge

B-2.6 Water Bath

B-3 PROCEDURE

B-3.1 Sample Preparation

To the weighed sample, add a little water and heat to 60°C. Allow to stand for some time. Add about 100 ml of 95 percent alcohol and centrifuge at 8 000 rpm for 10 min. Wash the residue with about 50 percent alcohol until filtrate gives negative test for sugars. Transfer the residue in a 500 ml conical flask with

about 100 ml of water. Add 20 ml of concentrated hydrochloric acid. Heat in boiling water bath for 2 h 30 min. Cool, neutralize with 1N sodium hydroxide using phenolphthalein as indicator and make up the volume to 500 ml. Take suitable aliquots and determine reducing sugar.

B-3.2 Negative Test for Sugar

Take few ml of filtrate in test tube. Add few drops of 10 percent alcoholic solution of alpha naphthol. Pour 1 ml of concentrated sulphuric acid to flow slowly down the side of test tube so as to form a layer beneath the aqueous solution. If sugar are present a red ring will appear within few seconds at the junction of two layers.

B-3.3 Estimation of Reducing Sugar by Dinitro Salicylic Acid Method (Colorimetric Method)

B-3.3.1 *Reagents*

a) *Dinitro salicylic acid (DNS) reagent* — Dissolve simultaneously 1 g of DNS, 100 g of crystalline phenol and 50 g of sodium sulphite in 100 ml of 1 percent NaOH by stirring. Store the reagent in a stopper bottle at 4°C. It is advisable to prepare the reagent without sodium sulphite and add it just before use.
b) *40 percent solution of Rochelle salt* — 40 g of sodium potassium tartarate in 100 ml distilled water.

B-3.3.2 *Apparatus*

Electronic balance, Spectrophotometer, Water bath, Test tube and Refrigerator

B-3.3.3 *Procedure*

Pipette 1 ml aliquot of the extract in a test tube and 1 ml of DNS reagent. Heat the mixture for 5 min in a boiling water bath. Add 1 ml of 40 percent Rochelle salt when the content of the tube are still warm. Cool the tubes under running tap water. Measure the absorbance at 575 nm. Calculate the amount of reducing sugar using glucose as standard.

B-3.3.4 *Preparation of Standard Curve of Glucose*

Prepare standard solution by dissolving 50 mg glucose in 100 ml water (0.5 mg/ml). For standard curve, take a series of tube containing 0.1 mg to 0.5 mg glucose (200-1 000 µl of standard solution). Adjust the volume to 1ml with distilled water. Develop colour using DNS reagent as described in **B-3.3.3**. Plot calibration curve of standard solution between concentration on X – axis and absorbance on Y – axis at 575 nm.

B-3.3.5 *Glucose in Hydrolyzed Sample*

Take suitable aliquot from hydrolyzed solution and develop colour as described in **B-3.3.3**. Calculate the amount of sugar present in hydrolyzed sample by putting the absorbance value in the standard graph on Y-axis and drawing a perpendicular line on X-axis.

B-3.4 Determination of Sugar Using Benedict's Quantitative Reagent (Titrimetric Method)

B-3.4.0 *Principle*

The cupric ion present in Benedict's reagent is converted as cuprous ion in presence of reducing substance (free aldehyde/ketone group of sugars). Complete conversion is indicated by end point of titration. The extent of reduction depends on the reducing power of the sugar.

B-3.4.1 *Reagents*

a) *Benedict's reagent* — Dissolve 173 g of sodium citrate and 100 g sodium carbonate in about 800 ml of warm water. Filter and make the volume to 850 ml. Dissolve 17.3 g of copper sulphate in about 100 ml of distilled water and make up to 150 ml. Pour the first solution into a 2 litre beaker and slowly add the copper sulphate solution with stirring.
b) *Standard glucose solution* — 0.5 g/100 ml.
c) Anhydrous sodium carbonate.

B-3.4.2 *Apparatus*

White porcelain basin or 100 ml conical flask, burette and pipette

B-3.4.3 *Procedure*

Measure 25 ml of Benedict's solution into a 100 ml conical flask. Add approximately 3 g of anhydrous sodium carbonate. Heat the mixture to boiling and when it is boiling vigorously, slowly run in the sugar solution from a burette. When a bulky white precipitate is formed, add the sugar solution more slowly. Continue the titration until the last trace blue or green has disappeared. Note the volume of sugar consumed. The burette reading for the titration of 25 ml Benedict's solution should be between 10 and 15 ml. If a smaller reading is obtained, the sugar solution should be diluted. If the reading is too large, then the solution must be concentrated. Repeat the titration with the hydrolyzed solution. Note the volume of hydrolyzed sample used for titration.

B-3.4.4 *Calculation*

25 ml of Benedict's reagent is equivalent to 50 mg of glucose. Therefore if the glucose solution gives a value of 12.5 ml then the solution contains 50/12.5 mg/ml or 4 mg/ml. The volume of hydrolyzed solution used for titration can be thus used for amount of glucose present per ml of solution.'

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Publication Unit, BIS, New Delhi, India